which will protect the test animal the same as the Standard Toxin-Antitoxin mixture. Test animals dying sooner than the controls indicate the unit value selected in that dilution was not present, whereas those living longer indicate a greater unit value.

[39 FR 16859, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974, and amended at 40 FR 760, Jan. 3, 1975; 40 FR 41996, Sept. 10, 1975; 43 FR 1479, Jan. 10, 1978; 50 FR 24905, June 14, 1985. Redesignated at 55 FR 35561, Aug. 31, 1990; 61 FR 51776, Oct. 4, 1996; 64 FR 43045, Aug. 9, 1999]

§ 113.452 Erysipelothrix Rhusiopathiae Antibody.

Erysipelothrix Rhusiopathiae Antibody is a specific antibody product containing antibodies directed against one or more somatic antigens of *Erysipelothrix rhusiopathiae*. Each serial shall be tested as provided in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) Each serial shall meet the applicable general requirements provided in § 113.450.

(b) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested using the two-stage test provided in this section.

(1) In the first stage, each of 40 Swiss mice, each weighing 16 to 20 grams, shall be injected subcutaneously with 0.1 ml of product (dried product shall be rehydrated according to label directions). Twenty-four hours postinjection, the injected mice and 10 additional mice designated controls shall be challenged subcutaneously with the same culture of *Erysipelothrix rhusiopathiae*.

(2) If less than eight of the 10 controls die from erysipelas within 7 days post-challenge, the test is invalid. All dead mice shall be examined to determine if the cause of death was *Erysipelothrix rhusiopathiae* infection.

(3) The mice injected with product shall be observed for 10 days postchallenge and all deaths recorded. The second stage shall be required when 7–10 of the mice injected with product die in the first stage. The second stage shall be conducted in a manner identical to the first stage.

(4) The results of the test shall be evaluated according to the following table:

Stage	Number of vaccinates	Cumulative number of vaccinates	Cumulative total number of deaths for a satisfactory test	Cumulative total number of deaths for an unsatisfactory test
1	40	40	6 or less	11 or more.
2	40	80	12 or less	13 or more.

[39 FR 16859, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974, as amended at 40 FR 20067, May 8, 1975; 40 FR 23989, June 4, 1975. Redesignated at 55 FR 35561, Aug. 31, 1990; 61 FR 51776, Oct. 4, 1996; 64 FR 43045, Aug. 9, 1999]

§ 113.453 [Reserved]

§ 113.454 Clostridium Perfringens Type C Antitoxin.

Clostridium Perfringens Type C Antitoxin is a specific antibody product containing antibodies directed against the toxin of *Clostridium perfringens* Type C. Each serial shall be tested as provided in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) Each serial shall meet the applicable general requirements provided in § 113.450.

(b) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested using the toxin-neutralization test for Beta Antitoxin provided in this section. Dried products shall be rehydrated according to label directions.

(1) When used in this test, the following words and terms shall mean:

(i) *International antitoxin unit.* (I.U.) That quantity of Beta Antitoxin which reacts with L_0 and L_+ doses of Standard Toxin according to their definitions.

(ii) *L_0dose.* The largest quantity of toxin which can be mixed with one unit of Standard Antitoxin and not cause sickness or death in injected mice.

(iii) *L_+dose.* The smallest quantity of toxin which can be mixed with one unit of Standard Antitoxin and cause death in at least 80 percent of injected mice.

(iv) *Standard antitoxin*. The Beta Antitoxin preparation which has been standardized as to antitoxin unitage on the basis of the International *Clostridium perfringens* Beta Antitoxin Standard and which is either supplied by or acceptable to Animal and Plant Health Inspection Service. The antitoxin unit value shall be stated on the label.

(v) *Standard toxin*. The Beta toxin preparation which is supplied by or is acceptable to Animal and Plant Health Inspection Service.

(vi) *Diluent*. The solution used to make proper dilutions prescribed in this test. Such solution shall be made by dissolving 1 gram of peptone and 0.25 gram of sodium chloride in each 100 ml of distilled water; adjusting the pH to 7.2; autoclaving at 250 °F. for 25 minutes; and storing at 4 °C. until used.

(2) The antitoxin content of the test sample shall be determined as follows:

(i) Make a dilution of Standard Antitoxin to contain 10 International Units of antitoxin per ml.

(ii) Make one dilution of Standard Toxin to contain 10 L_0 doses per ml and make a second dilution of Standard Toxin to contain 10 L_+ doses per ml.

(iii) Dilute 1 ml of the test sample with 49 ml of diluent and combine 1 ml of this dilution with 1 ml of the Standard Toxin diluted to contain 10 L_0 doses.

(iv) Combine 10 International Units of Standard Antitoxin with 10 L_0 doses of diluted Standard Toxin and combine 10 International Units of Standard Antitoxin with 10 L_+ doses of diluted Standard Toxin.

(v) Neutralize all toxin-antitoxin mixtures at room temperature for 1 hour and hold in ice water until injections of mice can be made.

(vi) Five Swiss white mice, each weighing 16–20 grams, shall be used for each toxin-antitoxin mixture. A dose of 0.2 ml shall be injected intravenously into each mouse. Conclude the test 24 hours post-injection and record all deaths.

(3) *Test Interpretation*. (i) If any mice inoculated with the mixture of 10 International Units of Standard Antitoxin and 10 L_0 doses of Standard Toxin die, the results of the test are inconclusive and shall be repeated: *Provided*, That, if the test is not repeated, the serial shall be declared unsatisfactory.

(ii) If less than 80 percent of the mice inoculated with the mixture of 10 International Units of Standard Antitoxin and 10 L_+ doses of Standard Toxin die, the results of the test are inconclusive and shall be repeated: *Provided*, That, if the test is not repeated, the serial shall be declared unsatisfactory.

(iii) If any mice inoculated with the mixture of Clostridium Perfringens Type C Antitoxin diluted 1:50 and 10 L_0 doses of Standard Toxin die, the antitoxin is considered to contain less than 500 International Unit per ml and the serial is unsatisfactory.

[39 FR 16859, May 10, 1974. Redesignated at 39 FR 25463, July 11, 1974. Redesignated at 55 FR 35561, Aug. 31, 1990, as amended at 56 FR 66784, Dec. 26, 1991; 61 FR 51777, Oct. 4, 1996]

§ 113.455 Clostridium Perfringens Type D Antitoxin.

Clostridium Perfringens Type D Antitoxin is a specific antibody product containing antibodies directed against the toxin of *Clostridium perfringens* Type D. Each serial shall be tested as provided in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) Each serial shall meet the applicable general requirements provided in § 113.450.

(b) *Potency test*. Bulk or final container samples of completed product from each serial shall be tested using the toxin-neutralization test for Epsilon Antitoxin provided in this section. Dried products shall be rehydrated according to label directions.

(1) When used in this test, the following words and terms shall mean:

(i) *International antitoxin unit*. (I.U.) That quantity of Epsilon Antitoxin which reacts with L_0 and L_+ doses of Standard Toxin according to their definitions.

(ii) *L_0dose*. The largest quantity of toxin which can be mixed with one-tenth unit of Standard Antitoxin and not cause sickness or death in injected mice.

(iii) *L_+dose*. The smallest quantity of toxin which can be mixed with one-tenth unit of Standard Antitoxin and